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COLLECTION AND PREPARATION OF MATERIAL FOR CLASSES IN ELEMENTARY ZOÖLOGY.

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DURING the past year the writer has frequently received inquiries concerning methods of collecting and preparing zoölogical material for class use. The purpose of the present article is to give some of the methods used in the Department of Zoölogy of the University of Michigan in supplying a class of over a hundred students with laboratory material for a first course in elementary invertebrate zoölogy. A few organisms are included that are not ordinarily studied in a beginners' course, as some of them may be found useful for demonstrations, or for supplementary work. On the other hand, no attempt is made to make the list comprise all the forms adapted to a first year's course; for many of these, directions for collecting would be superfluous.

1. *Amœba.*[1]— Collect the water plant Ceratophyllum, which will be found growing in the quiet water of ponds and rivers, in the same habitats with water lilies, Elodea, etc. Gather the Ceratophyllum in considerable quantities, packing it in pails

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[1] H. S. Jennings. "Methods of Cultivating Amœba and Other Protozoa for Class Use." *Journ. of Applied Micr.*, vol. 6, no. 7, p. 2406.

with enough water to cover. It is best to get material from several localities. If Ceratophyllum cannot be found, Elodea and various other plants may be used, but the results are less satisfactory.

Bring the material to the laboratory and there set up cultures as follows. Use "bacteria dishes" — shallow circular glass dishes, about 9 inches in diameter and 3 inches deep. Pack the material in these *firmly*, crushing it down. This will prevent growth of the plant, and favor fermentation. Pack to within an inch of the top of the dish, and add just enough water to cover the material. Cover the dishes with glass plates and put them in various warm and well lighted places about the laboratory. Each culture should be labeled with the *place* where the material was obtained, and the *date* when the culture was set up. Later, as organisms appear which are to be studied, their *names* should be added to the labels. *Label the dishes, not the covers.* If water evaporates so as to expose the plant material, enough should be added from time to time to make good the loss.

The first cultures should be started about *three weeks* before the Amœbæ are needed. A half dozen cultures should be started at this time and several new ones added every two or three days thereafter, until the class is nearly through studying Amœba. The time required for the cultures to mature varies; a single culture may last but a few days, and not all are successful — hence the necessity for frequent collections and many cultures.

The first indication of Amœba is a brown or a white scum appearing on the surface of the water. This consists mainly of bacteria, and the Amœbæ come to the surface to feed on them. Take up a bit of the scum with forceps, mount in a drop of water taken from the surface with a pipette, tease out the scum thoroughly with needles, and examine with a microscope. Amœbæ, if present, will usually be found among or near the particles of scum. Also, remove a bit of the plant material and scrape the slime from it. This will often contain more Amœbæ than the scum at the surface of the water. The specimens are small at first, but in a few days they become larger.

While the Amœbæ usually disappear from such a culture in a short time, occasionally they persist for a week or more, growing larger. In some cultures they seem to encyst or disappear almost entirely, and later reappear in considerable numbers. Hence old cultures should be watched, and retained until they become overgrown with fungi and hence worthless.

The advantages of the Ceratophyllum method, as described and used by Jennings, are that it gives Amœbæ in abundance, at the time desired, with almost absolute certainty. But the Amœbæ obtained by this method are seldom large; their development is often interfered with and they usually give place to other organisms before attaining maturity. Moreover, the presence of an excessive amount of bacteria and other organisms often obscures the Amœbæ, and makes it difficult to find and study them. To overcome these difficulties, the writer, aided by a suggestion from Professor Reighard, devised a modification of the above method which has proved a valuable supplement to it.

As soon as Amœba appears in the Ceratophyllum cultures, skim off the brown scum with a spoon, and put it in small bacteria dishes about four inches in diameter and one and one half inches deep, with enough water to fill the dish about one inch deep. Add a little of the decaying vegetable material from near the surface of the Ceratophyllum cultures. This should be done several days before the Amœbæ are desired for use. Cover the dishes and keep them in a *warm* place, but not in direct sunlight. In a few days Amœbæ will be found in abundance in some of these cultures, in the scum at the surface or in the ooze and on the decaying plant material at the bottom of the dish. Some of the cultures prepared in this way by the writer gave results far better than were obtained from the ordinary Ceratophyllum cultures; the specimens were remarkably large, active, and numerous. The Amœbæ pass through stages of development and sometimes practically all of them in a given culture encyst at the same time. They may reappear later in an active stage. Later on, among other organisms may appear in these cultures Arcella, Chilomonas, Paramœcium, Vorticella, and occasionally some colonial Protozoa.

2. *Arcella and Difflugia* will often be found in ordinary Amœba cultures, or in cultures made with dead water-lily leaves. In rather fresh cultures, these Protozoa may be found creeping along the surface of the vegetation. Take up a mass of the Ceratophyllum between the fingers and bruise it briskly against a watch glass; add a *little* water to the residue that clings to the glass. It will usually contain Arcella and Difflugia.

3. *Chilomonas* may appear at almost any time in Amœba cultures; but it is best to put up cultures especially for Chilomonas and Infusoria. Use Ceratophyllum and partly decayed water-lily leaves, and pack in bacteria dishes as for Amœba, but use less plant material and more water. Cultures prepared in this way may mature in a very few days. Mount a drop of the water containing Chilomonas, together with a little of the slime or decaying vegetable material by means of which the specimens may be entangled.

4. *Euglena* will appear in some of the cultures put up for Amœba, usually after the disappearance of Amœba, four or five weeks from the date of starting the cultures, but occasionally it appears before Amœba. It makes a solid deep-green (not blue-green) mass at the edge of the dish, especially on the side towards the light. A thick, pale brown, felt-like and granular scum on the surface of the culture seems to be favorable to the presence of Euglena. Such a scum contains delicate fungi, diatoms, desmids, Arcella, etc.

Euglena usually appears in only a few dishes, but in abundance, and lasts several days. Euglena is often associated with Phacus, which somewhat resembles it, but the latter organism is skate-shaped, and does not exhibit euglenoid movements.

Keeping the cultures away from a strong light may prevent the formation of an excess of chlorophyll in Euglena.

5. *Paramœcium.*— Prepare cultures in the same manner as for Chilomonas. Decaying water-lily leaves are especially good. The cultures require but a few days to mature, and last a long time. Mount a drop of water containing Paramœcia, together with a little of the decaying vegetable material about which they may collect.

6. *Vorticella* is often found on decaying duckweed, Riccia, and Ceratophyllum. Place a large handful of the material in a bacteria dish nearly filled with water. Vorticella often appears in cultures prepared for other purposes.

7. *Carchesium* often occurs on water snails and crustaceans amongst duckweed and Riccia. Collect in the same manner as Vorticella.

8. *Volvox* has been found to occur most abundantly amongst duckweed and Riccia, in pools or small ponds that do not dry up. Collect this material by dipping it up together with a little of the water and place a small handful of the material, with some of the water, in each of many bacteria dishes and add enough tap water so that the dishes are nearly full. Place the dishes so that one side is strongly exposed to light. After a few hours, specimens of Volvox may be seen with the naked eye, or with a hand lens, at the water's edge on the lighted side of the dish. Remove them with a pipette to a small covered dish filled with clean water. Volvox kept in clear water and strong light seeks the bottom of the dish, and can readily be found and removed with a pipette when wanted. If left in the original dish, there may be present small crustaceans which will eat them ; yet Volvox may sometimes be collected from these dishes for several days. Usually the material yields best after a day or two, and in three or four days becomes worthless. If obtained free from the animals that prey upon it, Volvox may be kept for several days or even longer.

Volvox is not always present in localities that seem favorable for it, and ponds that contain it in abundance are somewhat rare. In some situations it may be collected to advantage by sweeping a bolting-cloth net over water plants, or, better, using a "Birge net," which has a coarse net over the top for keeping out large forms and trash.

Volvox may be preserved in 4% formalin. Formalin specimens show flagella better than living ones. On account of the uncertainty of obtaining Volvox late in the fall, a supply of preserved specimens should be laid in early in the season.

In studying Volvox, mount the specimens in hollow slides or on ordinary slides with cover-glasses supported by fine glass rods or by bits of broken cover-glass.

9. *Pandorina* sometimes forms a green scum on the surface of the water amongst water lilies, or on the lily leaves.

10. *Hydra* occurs most abundantly on the following plants: duckweed, Riccia, Ceratophyllum, Elodea, watercresses. Two methods of collecting may be used. (a). Method best adapted to duckweed and Riccia: bring in the material with enough water to keep it moist. Fill a large number of bacteria dishes nearly full of clear water. Place in each dish a *small quantity* of the plant material — scarcely enough to cover the surface of the water on which it floats. Place each dish so that one side will be exposed to the light. A day or two later hydras will be found in the extended condition, clinging to the side of the dish, especially the lighted side, to rhizoids of plants, or to dead plants and sediment at the bottom. They may be removed from the sides of the dish with a pipette, or if clinging to plants, the latter may be removed with forceps. Place the hydras in a covered dish of clean water with a small amount of actively growing plant material. Here they will keep indefinitely and may readily be found when wanted. It is not safe to leave them in the original dishes, as there may be present crustaceans which will eat them, or an excess of vegetable matter may cause the water to become foul, which is unfavorable for Hydra. However, the dishes should be inspected daily, for sometimes the material will yield Hydra indefinitely.

(b). When Hydra is to be obtained from large plants like Ceratophyllum and Elodea, the best method is as follows: place a considerable quantity of the plant material in each of several large bacteria dishes and cover it with water. In a short time the water becomes foul through the decomposition of vegetable matter; the hydras then loosen their hold upon the vegetation and may be found floating at the surface of the water. They must be picked off at once with a pipette and removed to clean water.

Hydra will reproduce readily by budding if kept in a fairly warm room; but the optimum temperature is not very high. It is said that if Hydra is kept in the dark at a slightly lower temperature than usual for several days, this will favor the formation of spermaries and ovaries. Rarely, a mature fertilized egg in the winter condition may be found.

11. *Planarians* are found on the under side of stones in running water. They are usually abundant in a locality if found there at all. They may be removed from the stones by means of a thin wooden toothpick, and placed in a bottle of water for transportation.

12. *Earthworms.*—The *collection* of earthworms should be attended to as early in the fall as possible, as dry or cold weather may make it impossible to get them later. It is well to lay in a supply of preserved worms during the spring or summer. Specimens to be kept alive should perhaps not be collected until after the first of October, but it will be unsafe to wait much longer. Each student will need two or three preserved, and one or two living specimens.

The form used in the Zoölogical Laboratory of the University of Michigan is *Lumbricus herculeus* Savigny. The specimens range in length from about 6 to 11 inches, with an average of about 8 inches. They come out of their burrows on warm, rainy nights, usually lying extended with the posterior end of the body still in the entrance of the burrow. They are most abundant in old, rich gardens and lawns. Go out with a lantern and a pail after it has become quite dark; the harder it rains the better. By stepping lightly and not allowing the light to shine upon them too long, one may seize the worms with the hand. To catch them requires quickness and dexterity; but they must be pulled gently from the burrow.

When brought into the laboratory the worms that are to be preserved for dissection should be placed immediately in covered bacteria dishes (9 inches in diameter by 3 inches deep) between sheets of moist filter paper; the covers should be adjusted so as to admit a little air. Not more than twelve specimens should be placed in a dish, as they will soon die if large numbers of them are left together. The dishes should be kept in a cool place, avoiding direct sunlight. In the morning the paper should be changed, and injured or dead specimens removed; the operation should be repeated as often as is necessary. After a day or two the worms will have eaten enough moist filter paper to clean the alimentary canal of earth, and are ready for preservation.

Worms to be kept alive for a considerable length of time[1] may be placed, immediately after collection, between folds of moist muslin in bacteria dishes, with not more than a dozen specimens in a dish. Change or wash the cloth occasionally — at least every two weeks. The worms may be fed on leaves, etc., if desired. With proper care, they will keep in this way for months, and are always clean and ready for use at a moment's notice. The writer has found that the worms will keep quite as well if filter paper is used instead of muslin.

Preparation of Earthworms for Dissection.— Fresh specimens are too soft for convenient handling of the tissues during dissection; it is also inconvenient to keep a sufficient number of them alive for general dissection purposes; hence preservation is resorted to. Two methods are available: —

(*a*) *Chromic Acid Method.*

1. *Killing.*— Place in 4 % alcohol and gradually increase the strength to 8% in the course of the next two hours by adding stronger alcohol a few drops at a time. Wash in water to free from mucus and again immerse in 8 % alcohol until the worms are thoroughly stupefied and no longer contract when pinched with forceps.

2. *Fixing.*— Inject worms with 1 % chromic acid and immerse in the acid for about 4 hours.

The injection is best performed by means of a water pressure apparatus (see Fig. 1). This gives constant, uniform pressure. A head of about four feet of water should be used. A glass cannula may be made by drawing out the end of a piece of $\frac{1}{4}$ inch glass tubing to a very fine bore, and breaking it so as to leave a sharp point with a raw edge. The exact size must be determined by experiment. The stream may be allowed to flow continuously from the cannula. A pipette with point drawn out fine may be used in case this apparatus is not available, but it is not very satisfactory.

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[1] H. S. Jennings. "Keeping Earthworms Alive in Winter." *Journ. of Applied Micr.*, vol. 6, no. 7, pp. 2412–2413.

Fill a large shallow glass dish about $\frac{1}{2}$ inch deep with 1 % chromic acid. (Cover the hands and wrists thickly with vaseline while working with chromic acid.) Take each worm separately and lay it in an extended condition in the chromic acid, keeping it straight and seeing that it is not twisted. Inject immediately, about $\frac{1}{2}$ inch behind the clitellum, and again near the posterior end of the body if necessary. *Inject into the body cavity; be careful not to pierce the alimentary canal.* The worm should swell out slowly along its entire length and become very turgid. Too rapid injection may tear the tissues.

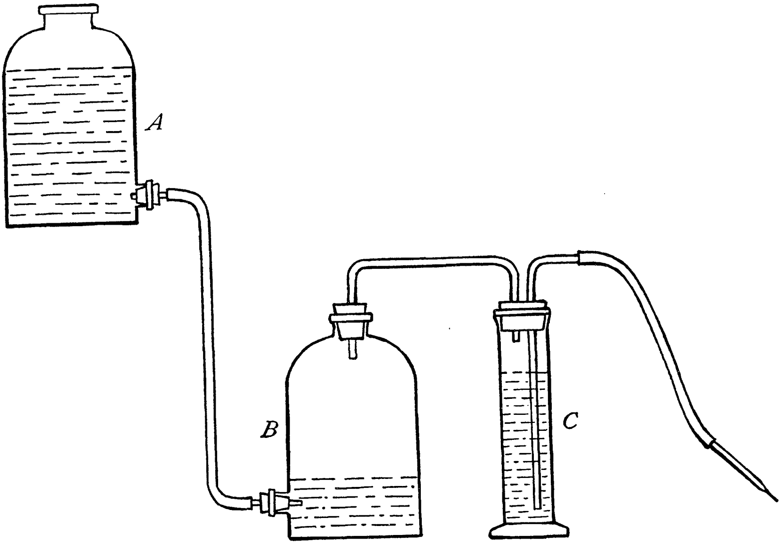

FIG. 1.— Constant pressure injection apparatus. *a* should be filled with water, part of which flows into *b*; *c* contains injection fluid.

The worms should be kept straight while in chromic acid. If left in the acid less than four hours they will eventually become too soft; if left in the acid much more than four hours they will be too brittle.

Wash thoroughly in running water until the yellow color is gone — about 12 hours. Be sure that the water reaches every part of the surface of each worm. If free acid remains on the worms it will make them brittle. The worms are a light drab, not yellowish brown, when well washed.

3. *Preserving.*— Place the worms in 50 % alcohol and leave for a day or two; the alcohol will become discolored by chromic acid. Change to 70 % and leave a day or two longer. Then change to the same grade (70%) for final preservation.

The alcohol will complete the process of removing free acid and will further harden the tissues. Stronger grades of alcohol than 70 % are not advisable for preservation, as they may cause the more delicate tissues to become brittle.

(*b*) *Alcohol Method.*

Kill as directed under chromic acid method. Place the worms in 50 % alcohol, keeping bodies as straight as possible. Leave 4 or 5 hours, then place in 70 % alcohol and leave over night. Next morning place in 96 % alcohol to harden them, and leave all day. Change to 70 % alcohol for final preservation.

Choice of Methods.— Specimens hardened in chromic acid are most satisfactory for general dissection purposes. The tissues are firm and leathery, and are not affected by water during dissection, hence they may be dissected under water instead of alcohol. This is a decided advantage. The largest and best marked specimens should be put up in this way.

The alcohol method of fixing is not so satisfactory for most purposes; the body wall and tissues generally are too soft to be conveniently handled unless the dissection is done under alcohol. But the method has the advantage of simplicity, and the smaller and more poorly marked specimens may be put up in this way for use in case other material runs short. Alcoholic specimens do very well for the study of the nervous system.

Fresh specimens are not only necessary for the study of the living worm, and for demonstrations of cilia, blood, cœlomic fluid, spermatozoa, etc., but are best for the study of the circulatory system and the nephridia.

For studying the circulatory system the living specimen may be stretched out in a dissecting tray and fastened with a pin through each end of the body; if desired it may first be stupefied with alcohol. The dissection should be carried on under normal salt solution (0.75 % NaCl). In the absence of living specimens, chromic-acid specimens do fairly well for the circulatory system.

For sectioning with a microtome it is necessary to have specimens with intestines entirely free from grit. Worms that have been kept in clean, moist muslin for a considerable length of time will usually be found to have the alimentary canal free from all undesirable substances. The muslin should be changed every day, and the worms must not be fed. A quicker method [1] is to flush the alimentary canal by means of an injection apparatus. Use a cannula with a rather large opening; the proper pressure must be determined by experiment. Stupefy the worms with dilute alcohol (3 to 6 %). Inject the alimentary canal from the posterior end with 0.75 % salt solution; roll the body of the worm back of the clitellum between the fingers and strip out the contents of the intestine. Then inject from the anterior end; this time the stream will go entirely through. Avoid too much pressure, as it will injure the tissues. Roll and knead the body while the stream is being started through. The fluid should be forced through the worm until it comes from the posterior end in a perfectly clear stream. The worm is then ready for fixing.

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[1] Raymond Pearl. *Journ. of Applied Micr.*, vol. 3, no. 1, p. 680.